NEUROGENESIS OF RESPIRATORY RHYTHM: A COMPUTATIONAL STUDY

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Abstract - **Results of computational modeling of the respiratory rhythm generation have been used for analysis of the conditions that may define a network-based vs. a pacemaker-driven rhythm-generating mechanism. The possible cellular and network factors contributing to the transitions between the network-based and pacemaker-driven oscillations are discussed in connection with experimental data obtained *in vivo*, *in situ* and *in vitro*.**

Keywords - **Respiratory rhythm, CPG, neurons, ionic channels**

INTRODUCTION

Two concepts were put forward to explain the generation of the respiratory rhythm in the brainstem. A *network* concept (based on *in vivo* studies) suggested that the rhythm is generated due to the network (mostly inhibitory) interactions between functionally different populations of respiratory neurons [1-3]. In contrast, a *pacemaker-based* or a *hybrid* concept (based mostly on *in vitro* data) suggested that a subpopulation of conditional pacemaker neurons comprises a rhythm-generating "kernel" providing a necessary bursting drive to a wider pattern-forming network [4,5]. The network theories and models have been successful in providing explanations to various systems-level phenomena, such as separate regulation of respiratory phase durations and various respiratory reflexes. However, the network concept requires the reciprocal inhibitory interactions between respiratory neurons and therefore has been unable to explain the pacemaker-driven rhythm recorded *in vitro*, which was proven to persist after blockade of synaptic inhibition [6]. The pacemaker-based theories and models face principal difficulties in providing explanations for the systems-level phenomena. In addition, the discharge pattern recorded *in vitro* differs in shape from the normal ("eupneic") breathing pattern, and appears to be similar to *gasping* [7].

During eupneic breathing, the firing frequency within inspiratory bursts, as assessed by activity of the phrenic nerve, increases in a "ramp-like" manner [1-3,7]. In contract, during gasping the phrenic discharges have a higher-amplitude decrementing pattern [7]. There is evidence, that eupnea and gasping are produced by fundamentally different neural mechanisms [7,8]. The bursts of rhythmic activity recorded *in vitro* also have a high-amplitude decrementing pattern.

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Both the rhythmic activity recorded *in vitro* [6] and that during gasping *in vivo* [9] persist after blockade of synaptic inhibition. This strongly supports the idea that, despite obvious differences in the physiological state, both the rhythmic activity observed *in vitro* and that during gasping are driven by a similar pacemaker-based mechanism operating in the rostral ventrolateral medulla (e.g. in the pre-Bötzinger complex (PBC)) [7, 10].

Alternatively, the eupneic respiratory rhythm may be disturbed by the blockade of synaptic inhibition [9,11]. Therefore, the network mechanisms (specifically, inhibitory interactions) appear to be necessary for the eupneic rhythm generation.

The current state of knowledge requires a comprehensive computational study of relationships between *in vivo* and *in vitro* data, and between rhythmogenesis mechanisms in eupnea vs. gasping.

Early computational models provided important insights into understanding the key network mechanisms for respiratory rhythmogenesis. However, they lacked intrinsic biophysical properties of neurons and hence did not allow for analysis of complex dynamic interactions between cellular and network processes underlying respiratory rhythm generation. Recently a new series of models has been developed using the Hodgkin-Huxley formal description of single neurons [4,5,10,12-15]. These models provided an opportunity to analyze the role of intrinsic neuronal properties in the respiratory rhythm generation. Specifically, our models [12,13] have clearly demonstrated that biophysical properties of single respiratory neurons contribute to the control of the durations of respiratory phases and phase transitions. Butera et al. [14,15] developed the first "realistic" models of conditional pacemaker neurons in the PBC that closely reproduced the generation of pacemaker-driven rhythm *in vitro*.

Finally, a consensus has been reached in understanding that the ponto-medullary respiratory network can generate rhythmic output by either a network or a hybrid pacemaker-network mechanisms depending on conditions [5,10]. If this is right than the next fundamental questions are: (1) *What are the conditions that define each of the mechanisms of rhythmogenesis?* (2) *How is the respiratory network re-organized to switch from one mechanism of rhythmogenesis to another?* (3) *Which mechanism of rhythmogenesis drives eupnea (i.e. normal breathing)?*

This paper presents the results of our attempt to address the above questions in computational modeling studies.

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Abstract

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MODELING THE CONDITIONAL PACEMAKER NEURONS

The previous models of pacemaker neurons developed by Butera et al. [14] closely captured the endogenous bursting activity recorded *in vitro*. However, these models contained only a minimal set of ionic currents necessary for bursting behavior and omitted transient potassium K_A^+, calcium and calcium-dependent potassium currents known to exist in respiratory neurons. The intrinsic bursting in these models was based on the activation of the persistent sodium current (Na_P^+). While developing our models, we used these models as a basis, but additionally incorporated the K_A^+ current and calcium (Ca_L^{2+}, Ca_T^{2+}) and calcium-dependent potassium ($K^+(Ca^{2+})$) currents.

Three distinct models of pacemaker neurons were developed [10]. They all contained the Na_P^+ current whose activation could potentially initiate bursting. The major difference between our models was the mechanism producing burst termination. In Model 1, burst termination was provided by slow inactivation of the Na_P^+ conductance (similar to Model 1 by Butera et al). In Model 2, this termination was provided by the activation of the slowly activating potassium current K_S^+ (similar to Model 2 by Butera et al.). In Model 3, the mechanism for burst termination was based on the high-threshold CaL^{2+} current (providing calcium influx and accumulation) and subsequent activation of the calcium-dependent potassium $K^+(Ca^{2+})$ current. Similar to the models of Butera et al., all of our models were able to generate endogenous busting activity under certain conditions [10].

ENDOGENOUS BURSTING ACTIVITY AND BALANCE OF IONIC CURRENTS

Incorporating additional potassium and calcium channels into the models allowed us to study their effects on neuronal firing behavior. We have found that the persistent sodium current (Na_P^+), which is supposed to play a major role in generation of endogenous bursting, has complex interactions with potassium currents (specifically with the K_A^+) [10]. As a result, at lower levels of $[K^+]$out (6 mM) the K_A^+ current suppressed the Na_P^+-dependent bursting behavior in all our models. The increase of $[K^+]$out to 9 mM reduced the K_A^+ current and hence released bursting activity in all models. Therefore, our modeling results allow the suggestion that suppression or release of endogenous bursting activity at the cellular level may be explicitly dependent upon the balance between the potassium currents and persistent sodium current.

It is known that *in vivo* severe hypoxia altered the eupneic augmenting pattern of integrated phrenic discharges to the decrementing bursts of gasping. Many data implicitly support the suggestion that hypoxia induces gasping through direct modulation of channel conductances and via alteration of ionic homeostasis in the extracellular environment. Specifically, it was shown that hypoxia suppresses several types of potassium channels [16-17] and activates the persistent sodium channels [18-20] in different areas of the brain. In addition, hypoxia induces the augmentation of the extracellular concentration of potassium [21], which also reduces the potassium currents.

Interestingly, the rhythmic activity demonstrated in vitro also requires an increase in extracellular potassium concentration up to 8-9 mM in order to be manifested [22,23]. Simple calculations based on the Nerst equation show that such an augmentation in extracellular potassium shifts the reversal potential for potassium (Ek) 10-20 mV to more positive values of voltage and reduces all potassium currents by about one half, even if the conductances of these channels remain unchanged.

The hypoxia-induced suppression of potassium currents and augmentation of persistent sodium current cannot of course be limited to a particular target area, such as the PBC. Rather, hypoxia influences most areas of the brain. However, recent studies have demonstrated that the PBC neurons have an extremely high intrinsic chemosensitivity to hypoxia and hence may play a specific role in rhythmogenesis of hypoxia-induced gasping [19,24]. The question of what properties of these neurons define their specific role in genesis of pacemaker-driven gasping-like oscillations is open for further experimental studies. One possible property, that distinguishes these neurons from other brainstem neurons, is a remarkably higher density of persistent sodium channels [25]. In addition, these neurons may have some potassium channels with higher sensitivity to oxygen, which may allow them to perform the function of central chemoreceptors [10,24].

EFFECTS OF PHASIC INHIBITION

In order to study the effects of phasic synaptic inhibition upon endogenous bursting activity, we applied a short-term synaptic inhibition to our models of conditional pacemaker neurons. We found that a short-term synaptic inhibition disturbed the endogenous bursting in all three models.

The hybrid pacemaker-network concept suggests that the PBC contains a subpopulation of conditional pacemaker neurons comprising a “kernel” of the respiratory CPG [4,5]. At the same time, the PBC appears to receive phasic inhibition from the rest of the ponto-medullary network, specifically from the pons [7,11,26] (note that the hybrid model by Smith et al. [5] also includes the inhibitory feedback from the rest of the network to the “kernel”). Our results allow the suggestion that during eupnea the phasic synaptic inhibition from other parts of the respiratory network (e.g. the pons) suppresses the pacemaker-driven oscillations in PBC, which therefore loses its “kernel” function and operates as a part of the respiratory network. This suggestion is consistent with the previous conclusions that the pons plays a necessary role in generation of the eupneic respiratory rhythm [7,11,26] and that the release of pacemaker-driven oscillations *in vitro* may occur partly because the pons is not present in most *in vitro* preparations. In addition, the release of hypoxia-induced gasping *in vivo* may also be connected with the hypoxia-dependent suppression of inhibition [27].

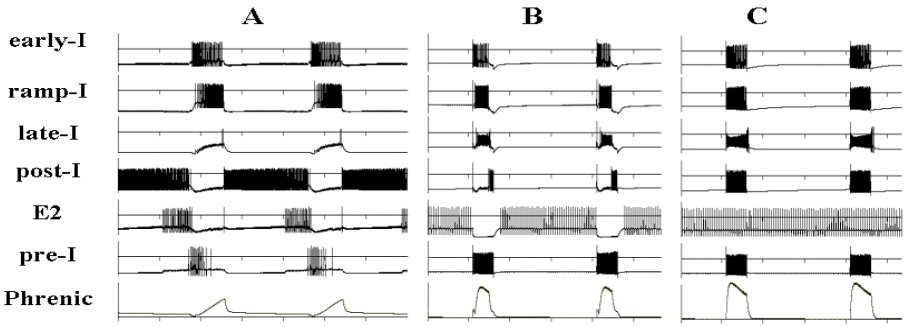

Fig. 1. Computer simulation of the transition in respiratory pattern generation from the network mechanism (the normal three-phase respiratory pattern in **A**) to a hybrid pacemaker-network mechanism (in **B** and **C**). All neurons in the model except E2 received excitatory drive from the conditional pacemakers. Changing the balance between potassium and persistent sodium current by an increase in $[K^+]$out switched rhythmogenesis from the network to hybrid pacemaker- network mechanism (**B**). The post-I and E2 neurons were still inhibited during inspiration by inspiratory neurons. An additional blockade of inhibition was applied to stimulate hypoxia-induced gasping (**C**). It produced a shift the discharge of post-I neurons to the inspiratory phase and eliminated the respiratory modulation of E2 firing. Note the changes in the shape of phrenic burst from an augmenting pattern during the network-based oscillations (**A**) to higher amplitude decrementing (gasping-like) pattern during the pacemaker-driven oscillations (**B** and **C**).

SWITCHING RESPIRATORY RHYTHMOGENESIS FROM A NETWORK TO A HYBRID PACEMEKER-NETWORK MECHANISM

Our theoretical analysis and computational modeling studies support the concept that the eupneic respiratory rhythm is generated as a result of the network interactions within the ponto-medullary neural network. We suggest that in eupnea the endogenous bursting activity in the PBC is suppressed by both the potassium currents (which overcome the bust-generating effect of the persistent sodium current) and the phasic inhibition from other parts of ponto-medullary network (specifically, from the pons). However, rhythmogenesis may be switched to a hybrid pacemaker-network mechanism (with a kernel in the PBC) under certain conditions. According to our modeling results, such conditions may include: (a) changing the balance between the potassium currents and persistent sodium current, allowing the latter to overcome the former, and (b) suppressing phasic inhibition to the PBC.

Therefore, according to our concept, the generation of the pacemaker-driven rhythmical activity *in vitro* may be explained by (1) artificial elevation of $[K^+]$out (typically used in *in vitro* preparations), which finally reduces the potassium currents, and (2) general reduction of inhibition in *in vitro* preparation and lack of inhibition from other parts of the network not present in these preparations (e.g. the pons).

Respectively, the release of hypoxia-induced (presumably also pacemaker-driven) gasping may be explained by a series of hypoxia-induced processes, such as: (1) suppression of the potassium and activation of the persistent sodium channels, (2) an increase in $[K^+]$out, and (3) general suppression of inhibition.

Our computer simulations at the network level [10] supported the possibility of the above transition (see Fig. 1). We used a modified version of our previous network model [13] and incorporated conditional pacemakers providing excitatory input to all types of respiratory neurons except the augmenting expiratory neurons (E2). An increase in $[K^+]$out from 6 to 9 mM released bursting behavior in the conditional pacemaker neurons and switched rhythm generation from the network to hybrid pacemaker-network mechanism. Importantly, our model reproduced the physiologically realistic changes in the shape of phrenic burst from an augmenting pattern during the network-based oscillations (Fig. 1A) to a higher amplitude decrementing (gasping-like) pattern during the pacemaker-driven oscillations (Fig. 1B and 1C).

Our experimental studies have provided additional support to our theoretical concept [28]. We used an *in situ* perfused preparation of rat as an experimental model to produce the transition from eupnea to *in vitro*-like oscillations. In our experiments, an increase of $[K^+]$out within the perfusate to 10 mM, in combination with 4-AP (a K_A^+ channel blocker) and a low dose of strychnine (to reduce synaptic inhibition), produced alteration of the regular augmenting pattern of phrenic nerve discharges to a decrementing pattern similar to that recorded *in vitro* (Fig. 2).

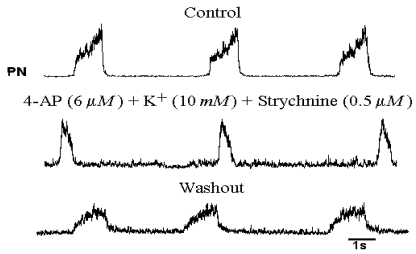

Fig. 2. The results of experimental studies. We used an *in situ* arterially perfused, decerebrate rat preparation. Phrenic nerve activity was recorded via a suction electrode. In order to experimentally verify our switching concept we added to the perfusate the following: 10 mM potassium, 4-AP to block K_A^+ channels, and a low dose of strychnine to antagonize glycine receptors. The effect was dramatic and changed the augmenting patterns of phrenic nerve activity (see "Control") to a decrementing gasping-like discharges similar to those recorded *in vitro* (middle trace). This effect was fully reversible following washout of the drugs and returning the $[K^+]$out to the control levels (see "Washout").

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